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Although documented methods are available for detecting and estimating aflatoxins in single commodities such as peanut products (1, 2), cottonseed products (3), coffee, cocoa, tea, copra and coconut, grains, etc. (4) no procedure is available that is specifically designed for application to mixed feeds. The reason is largely that mixed feeds are complex in nature, presenting unique analytical problems. The Association of Official Analytical Chemists recently recognized the importance of the feed area and has appointed Mr. Tom Romer of the Ralston Purina Co. as associate referee for mycotoxins in mixed feeds (4). I might add that we have maintained liaison with Mr. Romer on this problem.

Our interest in the feed area arises from our research program in maintaining and increasing feed markets for cottonseed and peanut meals. Both of these oilseed meals are used as protein supplements in many feeds, though not customarily in fish diets for the rapidly expanding fish-farming industry in the United States. For these purposes one must have an adequate analytical method to assure that both the oilseed meal, and the final mixed feed, are either free of or extremely low in aflatoxin content, since contamination can arise from any one of several feed components.

When starting this work we set as a goal a method capable of detecting aflatoxin contamination levels as low as 2–4 μg/Kg in typical mixed feeds. This level of contamination is probably of significance only in fish diets, since fish seem to be more susceptible than most species to the biological effects attributable to aflatoxins. For a review of the biological effects of aflatoxins, see a recent book, "Aflatoxins," edited by L. A. Goldblatt and published by Academic Press, New York.

We think we have achieved our original goal, though not with the acetonitrile:water extraction solvent given in your program abstract. That extraction solvent seemed to be adequate for peanut meals, and recoveries were good when pure aflatoxins were added to typical mixed feeds. Unfortunately, when acetonitrile: water extraction was applied to cottonseed meals, recoveries were low compared with the official AOAC method, which uses aqueous acetone for extraction (3). Aqueous acetone is not an ideal extraction solvent for use with feeds, for it removes too many interfering materials. On the basis of many extraction experiments, the system we finally selected involved prewetting of the feed sample with dilute acid, followed by extraction with methylene chloride (dichloromethane).

Table 1 lists the specified ingredients in a Bureau of Sports Fisheries Fish Diet, illustrating the multiplicity of ingredients in mixed feeds. As you can see, it contains a high proportion of fish meal, an ingredient we found to impose severe extract cleanup problems. Other mixed feeds, such as rabbit and poultry feeds, are equally complex.

Table 2 is a schematic flow sheet of the extraction step as applied to feeds. First, the sample is prewetted with 0.1*N* HCl, extracted with methylene chloride on a shaker, and filtered. The filtration is rapid. Although methylene chloride is similar to chloroform in extraction properties, we found it to yield cleaner extracts than did chloroform. It also requires no stabilizer and has a lower boiling point (40°C) than chloroform (60°C), which saves time in the evaporation steps.

An aliquot of the crude methylene chloride extract is then evaporated, dissolved in acetone, treated with lead acetate, made to a known volume, and refiltered. The lead acetate treatment removes chlorophyll(s), lipid and fatty acid contaminants, and other unknown materials which tend to streak badly on thin-layer plates.

An aliquot of the filtrate is partitioned into methylene chloride to separate aflatoxins from residual lead acetate and soluble lead pigment derivatives. After evaporation, it is dissolved in about 10 ml of methylene chloride and applied to the chromatographic cleanup column.

If one were interested in screening feeds for gross aflatoxin contamination, the semipurified extract after lead acetate treatment could be dissolved in benzene:acetonitrile and applied directly to a TLC plate. This is adeqate for B_1, but there will usually be interferences in the B_2–G_1 R_f area.

As shown in Table 3, when one applies the extraction to oilseeds, oilseed meals, and grains, an aliquot of the crude methylene chloride extract is transferred directly to the cleanup

TABLE 1. Composition of fish diet.

Specifications, fish food diet PR-4[a]			
Ingredient	%	Ingredient	%
Herring meal	33-35	Delactosed whey	4
Corn gluten meal	6	Dehyd. alfalfa	3
Wheat middlings	14-22	Mineralized salt	2
Corn fermentation extr.	8	Vitamin premix no. 21	4
Soybean meal	10	Fish oil	4
Yeast	4		

Crude protein, 40%; crude fiber, 5%.
Fish meal protein, 24.5%; moisture, 10.5%.
Crude fat, 7–8%.

[a] Dept. of Interior, Bureau of Sport Fisheries and Wildlife Specifications SFW 4-9.

column. Lead acetate treatment is not required for such extracts.

The chromatographic column used (Table 4) is a standard 400 × 20-mm-ID tube with a coarse fritted disc. We added Teflon stopcocks, which are useful when one operates 5 to 6 columns simultaneously.

A small pad of glass wool is placed over the fritted disc, the column is filled about 1/3 full with methylene chloride, and 5 g of acidic alumina, 80–200-mesh, is poured in and allowed to settle. Then, 15 g of silica gel (Mallinckrodt cc-7, 100–200-mesh) is slurried in methylene chloride and poured into the column. After settling, the column is topped with 3 cm of anhydrous granular sodium sulfate. It takes about 5 minutes to prepare a column.

The semipurified extract, dissolved in *ca.* 10 ml of methylene chloride, is transferred to the column, and the column is washed with:

a) 100 ml of benzene:acetic acid (9:1)
b) 150 ml of ether:hexane (3:1).

Both washes are discarded.

TABLE 2. Aflatoxins in mixed feeds.

I. Extraction and lead acetate cleanup

50-g smpl. + 70 ml 0.1*N* HCl + 10 g filter aid
Add 250 ml methylene chloride; shake 30 min; filter
Evap. 100 ml filtr; dissolve in 20 ml acetone; add 60 ml H_2O and 20 ml lead acetate (20%) soln.
Add 5 g filter aid; stir; filter
Shake 50 ml filtr. with 50 ml M. C. (2 ×) in separatory funnel
Evaporate M. C. phase to near dryness
Dissolve in 10 ml M. C.; to chromatographic column

Aflatoxins are eluted with 200 ml of methylene chloride:acetone (75:25). Total time for the two washes and aflatoxin elution is about 60 min.

The dual acidic alumina–silica gel column yields cleaner final extracts of mixed feeds than silica gel alone. It holds gossypol derivatives tenaciously thus allowing crude methylene chloride extracts of cottonseed and cottonseed meals to be applied directly to the column.

The benzene:acetic acid wash is most effective for removing many interfering materials in extracts of feeds, oilseeds, and oilseed meals. This solvent was originally recommended by Nesheim (5) as a TLC developer for ochratoxins, and by Eppley for eluting ochratoxins from silica gel columns (6). It does not strip aflatoxins.

The ether:hexane wash removes excess benzene:acetic acid, and also several blue fluorescent non-aflatoxin materials not removed by the benzene:acetic acid.

Methylene chloride:acetone (75:25) quantitatively strips aflatoxins and produces cleaner extracts than either chloroform:acetone or chloroform:methanol elution solvents.

Thin-layer chromatography (Table 5) is conducted on plates coated with Adsorbosil-1,

TABLE 3. Assaying Aflatoxins in oilseeds, oilseed meals, grains.

I. (A) Extraction

50-g sampl + 70 ml 0.1*N* HCl + 10 g filter aid
Add 250 ml methylene chloride; shake 30 min; filter
50 ml filtrate directly to chromatographic column

Table 4. Assaying aflatoxins in oilseeds, oilseed meals, grains, feeds.

II. Chromatographic column

Column: 400 mm × 20 mm; coarse disc
Adsorbent(s): 5 g acidic alumina
15 g CC-7 (100–200-mesh) silica gel
Extract from I or I (A) in M. C.; apply to column
Wash with 100 ml benzene: HAC (9:1); discard
Wash with 150 ml ether:hexane (3:1); discard
Elute aflatoxins with 200 ml M. C.:acetone (75:25)
Evaporate to dryness
To thin-layer chromatography

developed in chloroform:acetone (9:1) in a Thomas-Mitchell steel tank, essentially as specified in the official AOAC methods for aflatoxins in peanut products (1, 2). We modified the development by placing distilled water in a trough at the front of the tank, and the development solvent in another trough at the back of the tank, with the gel layer on the plate facing the maximum tank volume. This is a modification of Stubblefield's chloroform:acetone: water (88:12:1.5) development mixture (7), and we find it gives excellent resolution of aflatoxins.

Both preliminary and quantitative estimation of aflatoxins on TLC plates (Table 6) are conducted as outlined in the official AOAC method for aflatoxins in cottonseed products (3). Any established quantitative technique would be equally satisfactory. All of the data we report were obtained from fluorodensitometric scans of thin-layer plates.

A useful aflatoxin confirmation test is to spray the plate, after quantitation, with 25% sulfuric acid as recommended by Von Schuller and others (8). Aflatoxins B_1 and B_2 turn strongly yellowish fluorescent, while G_1 and G_2 turn to a bluish-yellow fluorescence after the acid spray. The color change can be detected with as little as 1 ng/spot. This color change after spraying is indicative but not conclusive identification, although failure of the color change immediately rules out any presumptive aflatoxins.

Since aflatoxins B_1 and G_1 are easily converted to the hydroxy derivatives B_{2a} and G_{2a} in the presence of strong acid, we were at first concerned about adding to the sample 0.1*N* HCl, which has a pH of 1.1.

The data in Table 7 show that the buffering effect of a 50-g sample prevents any marked pH change in the media. A sample:water slurry of pH 6.2 only changes to 5.1 in the presence of the hydrochloric acid. The other wetting agents, 2% KH_2PO^4 and 1*N* acetic acid, were used in exploratory work on the extraction of aflatoxins from cottonseed meals.

The extraction data outlined in Table 8 are typical of the problems we encountered with contaminated cottonseed meals. With this meal (204), the official AOAC method, shown at

Table 5. Assaying aflatoxins in oilseeds, oilseed meals, grains, feeds.

III. Thin-layer chromatography

Plates: 20 × 20 cm
Silica gel:adsorbosil-1 (500-micron); activate 105°C
Tank: Thomas-Mitchell; stainless-steel
Solvent: distilled water, front trough
$CHCl_3$:acetone (9:1), back trough
Development: 12 cm beyond origin, *ca.* 50 min (25°C)

TABLE 6. Assaying aflatoxins in oilseeds, oilseed meals, grains, feeds.

IV. Preliminary and quantitative TLC

Dissolve purified extract in benzene:acetonitrile (98:2)

Preliminary TLC

Quantitative TLC
Visual or densitometric

As outlined in: J.A.O.C.S. *45*, 694-699 (1968)
J.A.O.A.C. *52*, 405-409 (1969)

Confirmation: Spray plate with 25% H_2SO_4 in H_2O
Aflatoxins B_1, B_2, G_1, G_2 turn yellow fluorescent

the bottom of the Table, gave 140 μg/Kg of B_1, 17 μg/Kg of B_2 and 157 μg/Kg of total aflatoxins (B_1 and B_2). G aflatoxins are rarely found in cottonseed meals. Extraction with acetonitrile:water, 85:15 or 80:20, yielded about 40% recovery of aflatoxins, and addition of H_3PO_4 to the solvent increased recovery to about 67%.

Wetting the sample with water and extracting with methylene chloride gave 64% recovery. Wetting the meal with 2% KH_2PO_4 solution (pH 4.5) prior to methylene chloride extraction increased recovery to 90%.

The best recovery was obtained by wetting the meal with 70 ml of 0.1*N* HCl prior to methylene chloride extraction. As Table 8 shows, the values are equivalent to those with the official method. We are checking this phenomenon with peanut meals, and preliminary results suggest that the extraction problem is not as critical as it is with cottonseed meals.

Table 9 summarizes comparative analyses of eight contaminated cottonseed meals by the proposed mixed-feed method and the AOAC official method. As Table 9 shows, the two procedures are in good agreement for both high and low levels of contamination.

Table 10 outlines typical recovery experiments in which pure aflatoxin B_1 was added to feeds at levels from 10 to 50 μg/Kg. Recovery was adequate. We feel that recovery from spiked samples is useful for establishing absence of interferences, and for giving assurance that aflatoxins are not degraded. This type of recovery should not be used as the sole criterion of the accuracy of a given method, for the real problem lies in the recovery from a naturally contaminated product.

TABLE 7. pH experiment, cottonseed meal.

Weight of meal (g)	Aqueous solution added	pH of meal slurry[a]
50	70 ml H_2O	6.2
50	70 ml 2% KH_2PO_4	5.9
50	70 ml 1*N* HAC	4.3
50	70 ml 0.1*N* HCl	5.1

[a] Glass-calomel electrode.

Table 11 outlines recovery experiments from contaminated cottonseed meals added to typical mixed feeds. In each case the meal alone was analyzed by the proposed method, and the

TABLE 8. Extraction of aflatoxins, cottonseed meal 204

Extraction solvent	Aflatoxins (μg/kg[a])		
	B_1	B_2	Total
Acetonitrile:H_2O (85:15)	67	8	75
Acetonitrile:H_2O (80:20)	62	12	74
Acetonitrile:H_2O (85:15) + 0.5% H_3PO_4	92	19	111
Methylene chloride + 35 ml H_2O	83	20	106
Methylene chloride + 75 ml H_2O	85	15	100
Methylene chloride + 75 ml H_2O + NaCl	71	9	80
Methylene chloride + 75 ml 2% KH_2PO_4	133	28	161
Methylene chloride + 70 ml 0.1*N* HCl	148	28	176
Official A.O.A.C. method (3)	140	17	167

[a] Densitometric analysis.

amount in the mixtures was calculated from these values and the weight of the cottonseed meal used. These are shown in the two columns labeled "calculated B_1 and B_2."

The last two columns give values obtained from analysis of the feed-cottonseed meal mixtures. Recovery values were adequate for B_1 levels down to about 4 µg/Kg, and for B_2 down to 1–2 µg/Kg.

Similar recovery experiments are under way on the addition of peanut meals to typical feeds. Preliminary results suggest that recovery of B_1 and B_2 is good although some feeds produce streaking at the R_f of G_1–G_2. This is no insurmountable problem in visual analysis, but does create problems in fluorodensitometric scans of the plate.

In summary, the method proposed seems satisfactory for estimating aflatoxin B_1 and B_2 levels as low as 4–5 µg/Kg in most mixed feeds. Evidence of freedom from interferences in the G_1–G_2 R_f area on TLC plates must await completion of experiments now in progress.

Reagents

(a) *Solvents:* ACS grade, methylene chloride (dichloromethane); diethyl ether (anhydrous, ≤ 0.01% ethanol); hexane (BR 68-69°C); acetone; benzene; chloroform (≤ 0.75% ethanol); acetic acid (glacial); hydrochloric acid (36.5-38.0% HCl).

(b) *Dilute HCl (0.1N)*—Dilute 8.9 ml conc. HCl to one liter with distilled water.

(c) *Lead acetate solution* (20%)—Dissolve 200 g neutral lead acetate trihydrate in distilled water, add 3 ml glacial acetic acid, dilute to 1 liter.

TABLE 9. Comparison of two methods, aflatoxin in cottonseed meals.

Meal no.[b]	Aflatoxin content (µg/kg[a]) Mixed-feed method B_1	B_2	Total	Official AOAC method[c] B_1	B_2	Total
1	29	6	35	21	2	23
2	95	15	110	92	20	114
3	108	26	134	102	25	127
4	134	17	151	142	22	164
5	151	31	182	132	16	148
6	512	122	634	510	112	622
7	527	81	608	558	110	668
8	638	100	738	624	117	741

[a] By densitometric analysis.

[b] Mold-damaged meals

[c] J.A.O.A.C. *52*, 405-409 (1969).

TABLE 10. Recovery of aflatoxin B_1 added to mixed feeds.

Type of mixed feed	Aflatoxin B_1 (µg/kg) Added[a]	Found[b]
Rabbit chow	50	46
Fish diet A	50	47
Fish diet B	50	50
Fish diet B	20	18
Fish diet B	10	12

[a] Pure B_1 added.

[b] By densitometric analysis.

(d) *Filter aid*—Johns-Manville Celite analytical filter aid.

(e) *For column chromatography*—

(1) Sodium sulfate, anhydrous, granular.

(2) Silica gel, Mallinckrodt cc-7, 100–200-mesh. Dry 2 hr. @110°C; add 1% H_2O.

(3) Acidic alumina, Fisher A-948, 80–200-mesh, Brockman activity grade I, pH 4.1–4.2; or E. Merck 1078, distributed by Brinkmann Instruments Inc., Catalog No. 68-01-29.

(4) Benzene:acetic acid wash solvent. Mix 9 volumes of benzene and 1 volume of glacial acetic acid.

(5) Ether:hexane wash solvent. Mix 3 volumes of anhydrous ether and 1 volume of hexane.

(6) Aflatoxin elution solvent. Mix 7.5 volumes of methylene chloride and 2.5 volumes of acetone.

TABLE 11. Recovery of aflatoxins in cottonseed meal added to mixed feeds.

Type of mixed feed	Mixture (g) Mixed feed	Meal	Aflatoxins (µg/kg) Calculated[a] B_1	B_2	Found B_1	B_2
Rabbit chow	40	10	22	5	19	7
Fish diet A	40	10	19	3	19	4
Fish diet B	40	10	22	5	18	5
Fish diet C	40	10	22	5	21	8
Fish diet D	40	10	22	5	22	8
Fish diet D	45	5	11	3	12	3
Fish diet D	48	2	4	1	5	2

[a] Based on analysis of cottonseed meal alone, and wt. used in mixture, densitometric analysis.

(f) *For thin-layer chromatography*—

(1) TLC spotting solvent. Benzene: acetonitrile (98:2 v/v).

(2) Silica gel, Adsorbosil-1, Applied Science Labs, Inc. Shake 50 g silica gel with 75 ml of distilled water for *ca.* 1 min. Immediately coat 5 plates with a 500-micron (0.5-mm) wet thickness layer. Air-dry *ca.* 30 min, activate *ca.* 2 hr at 105–110° C, store in desiccator over drierite or silica gel desiccant.

(3) Development tank. Thomas-Mitchell stainless steel, equipped with two troughs (A. H. Thomas).

(4) Development solvent. Chloroform: acetone (9:1 v/v).

(g) *Aflatoxin standards:* Prepare from individual pure crystalline aflatoxins B_1, B_2, G_1 and G_2 of primary standard quality (9) dissolved in benzene:acetonitrile (98: 2v/v), and suitably mixed and diluted to contain 1.0 μg/ml each of B_1 and G_1 and 0.3 μg/ml each of B_2 and G_2.

Equipment

(a) *Chromatographic tubes:* 400-mm-long × 20-mm-I.D., with coarse-porosity fritted disc (Corning 3845 or Kimble 28570); tubes are preferably fitted with a Teflon stopcock.

(b) *Mechanical shaker:* Burrell "Wrist Action," or equivalent, fitted to hold 500-ml Erlenmeyer flasks.

(c) *Viewing cabinet;* visual analysis: Ultraviolet Products Model C-4 or C-6, or equivalent, equipped with two long-wave UV lamps and contrast filter.

(d) *Densitometer; fluorodensitometric analysis.* As described in reference (3). Other suitable densitometers can be substituted.

(I) Sample Preparation

(a) *Feeds:* Grind granular or pelleted feeds in a Wiley, or equivalent, mill to pass a 2-mm screen.

(b) *Oilseeds and oilseed meals:* Grind as in (a) above.

(II) Sample Extraction

(a) *Feeds:* Weigh a 50-gram well-ground sample into a 500-ml Erlenmeyer flask, add 70 ml of 0.1*N* hydrochloric acid, and shake to wet entire sample. Add *ca.* 10 g of Celite filter aid and 250 ml of methylene chloride. Stopper with a leak-proof polyethylene or glass stopper, shake vigorously for 30 minutes on a mechanical shaker. Filter through a folded 18.5-cm circle of Whatman No. 4, or equivalent, paper in a suitable funnel.

(b) *Oilseeds, oilseed meals, grains, fish meal:* Extract as in (a) above.

(III) Preliminary Purification

(a) *Feeds:* Measure 100 ml of crude extract from II (a) above into a clean 250-ml beaker and evaporate to near dryness on a steam bath, avoiding overheating of the dry extract. Dissolve in 20 ml of acetone, add 60 ml of distilled water, and 20 ml of lead acetate solution. Stir and let stand for *ca.* 5 minutes until coagulation occurs. Add *ca.* 5 g of Celite filter aid, stir, and filter through Whatman No. 4, or equivalent, paper. Measure 50 ml of the filtrate into a 250-ml separatory funnel and extract with 50 ml of methylene chloride, shaking vigorously for *ca.* 1 minute. When the layers separate, drain the methylene chloride (lower) layer through a *ca.* 2-inch column of anhydrous granular sodium sulfate in a Butt, or equivalent, tube, collecting filtrate in a clean 250-ml beaker. Repeat extraction with a second 50-ml portion of methylene chloride. Evaporate the filtrate to near dryness on a steam bath, and dissolve in *ca.* 10 ml of methylene chloride.

(b) *Oilseeds, oilseed meals, grains, fish meal:* Preliminary purification of the extract with lead acetate is not required. Transfer 50 ml of the crude methylene chloride extract from II (b) above directly to the chromatographic column below.

(IV) Column Chromatography

(a) *Column preparation:* Place a small glass-wool pad over the fritted disc of a chromatographic tube, and tamp gently. Fill the column *ca.* one-third full with methylene chloride, and pour in 5 g acidic alumina. Slurry 15 g of cc-7 silica gel (100–200-mesh) in a 100-ml beaker with *ca.* 40 ml of methylene chloride, and pour into the tube. When the gel settles, top carefully with a *ca.* 2-cm layer of anhydrous granular sodium sulfate, and drain the column to just above the sodium sulfate layer.

(b) *Feed extracts:* Transfer the semi-purified feed extracts from III (a) above to the column, washing the sample beaker with *ca.* 5 ml of methylene chloride to effect quantitative transfer.

When the methylene chloride solution of the sample just passes through the top of the sodium sulfate, wash the column successively with 100 ml of the benzene:acetic acid, and 150 ml of the ether:hexane wash solutions, allowing the first wash solvent to just pass through the top of the sodium sulfate before adding the second. Discard both column washes. Elute aflatoxins with 200 ml of the methylene chloride:acetone (75:25) elution solvent, collecting the eluate in a clean 250-ml beaker. Evaporate the solvent to near dryness on a steam bath, transfer quantitatively to a 2-dram vial, washing the beaker with a fine stream of methylene chloride to effect transfer. Evaporate to dryness under a gentle stream of nitrogen, and reserve for thin-layer chromatography.

(c) *Extracts of oilseeds, oilseed meals, grains, etc.:* Transfer 50 ml of the crude methylene chloride extract from II (b) above (omitting the lead acetate treatment) directly to the chromatographic column, and treat exactly as in IV (b) above.

(V) Thin-Layer Chromatography

(a) *Preliminary TLC:* This step can be omitted if approximate aflatoxin content is known. Dissolve the purified sample extract from IV (a) or (b) above in 0.5 ml of benzene:acetonitrile (98:2), and shake vigorously for *ca.* 1 minute on a Vortex, or equivalent, tube shaker. Spot aliquots of 2, 5, 5, and 10 μl of sample extract and 1, 3, and 5 μl of the aflatoxin standard on a TLC plate, placing spots *ca.* 1 cm apart on an imaginary line *ca.* 4 cm from bottom of plate. Spot 5-μl of aflatoxin standard on top of one of the 5-μl sample spots as internal standard. Remove *ca.* 0.5 cm of gel from side edges of plate and scribe a line *ca.* 12 cm from origin as a solvent stop. Place sample troughs touching the front and rear walls of a dry Thomas-Mitchell stainless-steel tank. Add *ca.* 40 ml of distilled water to the front trough, and 50 ml of chloroform:acetone (9:1) to the rear trough. Place the plate upright in the rear trough, facing the gel layer toward the maximum tank volume. Cover the tank and develop for 12 cm of solvent travel (*ca.* 50 min at 24–25°C). Estimate approximate aflatoxin content as outlined in reference (3).

(b) *Quantitative TLC:* Conduct quantitative visual or fluorodensitometric thin-layer chromatography as outlined in reference (3) except that benzene:acetonitrile (98:2) is used as the spotting solvent, and thin-layer development is conducted as outlined under Preliminary TLC above. Final purified extracts from IV (b) or (c) above represent 10 g of original sample. Calculate aflatoxins accordingly as outlined in reference (3).

(VI) Aflatoxin Confirmation

After preliminary or quantitative TLC has been conducted, spray the plate gently with a 25% aqueous solution of sulfuric acid, and view under long-wave UV light. Aflatoxins B_1 and B_2 turn from a blue to bright yellow fluorescence, while G_1 and G_2 turn from bluish-green to bluish-yellow fluorescence. The color change is sensitive down to about 1 ng/spot. The color change is indicative of, but not absolutely conclusive evidence of, aflatoxins, whereas failure of unknown spots to exhibit the change in fluorescence immediately rules out presumptive aflatoxins.

Notes

1. If methylene chloride is not available, chloroform ($\leq$ 0.75% ethanol) can be substituted, but methylene chloride is the preferred solvent.
2. Acidic aluminas other than those specified may be equally satisfactory. Alkaline aluminas should not be used.
3. In addition to mixed feeds, the procedure is applicable to all peanut and cottonseed products, to other oilseeds and oilseed meals, copra and coconut, grains, and fish meals. For these applications, a 50-ml aliquot of the crude methylene chloride extract is applied directly to the chromatographic column, omitting the lead acetate purification step under III (a) above.

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